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[COMMUNICATION]

T Cell-specific XTLA-1 Antigens from *Xenopus laevis* Tadpole and Froglet are not Identical

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ABSTRACT—T cell-specific XTLA-1 antigen molecules were immunoprecipitated from tadpoles and froglets of J strain *Xenopus laevis*, and analysed by gel electrophoresis. Both the tadpole and froglet XTLA-1 molecules have an apparent molecular size of 120 KD as analysed by SDS-PAGE. But, after deglycosilation with endo F glycosidase, the froglet XTLA-1 molecules show more extensive charge heterogeneity than the tadpole ones do on two-dimensional gels. The results suggest that the XTLA-1 molecules partially changes their structure during metamorphosis.

INTRODUCTION

During amphibian metamorphosis, profound biochemical transitions occur in association with the morphological and physiological changes. In erythrocytes, for example, there is a complete switch in hemoglobin from larval to adult type molecules. It has been demonstrated that such a switch in hemoglobin types results from a replacement in the blood of larval hemoglobin-producing erythrocytes by newly differentiating adult hemoglobin-producing cells [1]. Recently, evidences have been accumulated in *Xenopus* supporting that similar biochemical and functional transitions may occur in association with the shift of lymphoid cell types during metamorphosis (see review [2]).

The XTLA-1 antigen recognized by a mouse monoclonal antibody is the only thymus-dependent (T) cell-specific surface antigen that has so far been identified in *Xenopus*, and provides a useful marker for studying the development, differentiation and function of the T cell system of this animal [3–5]. The XTLA-1 molecules immunoprecipitated from froglet thymocytes and splenocytes are glycoproteins of an apparent molecular size of 120 k dalton (KD) [5]. In the present study, the biochemical characterization was carried out on the XTLA-1 molecules immunoprecipitated from tadpoles as well as froglets. The results indicate that the tadpole XTLA-1 molecules have the same apparent molecular size as the froglet ones but differ in a charge heterogeneity, suggesting that the XTLA-1 molecules may partially change their structure during metamorphosis.

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MATERIALS AND METHODS

Major histocompatibility complex (MHC) homozygous, partially inbred J strain *Xenopus laevis* were used. Mature females were injected with 300 U of human chorionic gonadotropin (Gonatropin 1000; Teikoku Zoki Co.) to induce ovulation, and the eggs obtained were artificially fertilized in Steinberg's solution according to the method described previously [6]. Embryos and larvae were kept in aquaria and their developmental stages were determined by the Normal Table of Nieuwkoop and Faber [7]. The mouse monoclonal antibody, XT-1, was produced by the previously described method [5] and the IgG fraction of the hybridoma ascites, obtained by the affinity chromatography on a protein A-Sepharose CL-4B (Pharmacia) column, was used for immunoprecipitation.

Thymuses were dissected from tadpoles between stages 55–56 (35 days after the fertilization) and from froglets of 8 months in age, and the thymocyte suspensions were prepared in amphibian phosphate buffered saline. Cells from 50 tadpoles or 10 froglets were pooled and labeled with ^{125}I. Cell labeling, immunoprecipitation, digestion with endo F glycosidase (glycopeptidase F-free; Boehringer Mannheim Biochemica) and gel electrophoresis were carried out exactly as described previously [5].

RESULTS AND DISCUSSION

The lysates of radioiodinated thymocytes from tadpoles and froglets were immunoprecipitated by sequential incubations with the specific monoclonal XT–1 antibody and protein A-Sepharose CL–4B beads, and analysed by SDS–PAGE. The results showed that both tadpole and froglet XTLA–1 molecules run as a single band around an apparent molecular size of 120 KD (Fig. A), confirming the previous results on the adult molecules [5].

Since the endo F glycosidase-treated XTLA–1 molecules from froglet thymocytes were known to segregate into the characteristic pattern of spots on O'Farrell's two-dimensional gel electrophoresis, immunoprecipitates from tadpoles and froglets were digested with endo F glycosidase and then analysed by two-dimensional gel electrophoresis. As shown in the previous study [5], the deglycosilated froglet XTLA–1 molecules formed a number of spots (some are fused) with a slightly reduced molecular size near the acidic end of the gel (Fig. B). In contrast, the tadpole XTLA–1 molecules migrated into a more restricted charge heterogeneity under the same conditions, i.e., several relatively basic spots seen on the gel of the froglet antigen were not detected on that of the tadpole antigen. Endo F glycosidase was reported to remove both high-mannose and complex type glycans linked through asparagine to the peptide backbone [8]. The difference in charge distribution patterns between the tadpole and froglet XTLA–1 molecules observed here is, therefore, assumed to represent a difference in other linked glycans and/or amino acid compositions of the 120 KD glycopeptides.

During the metamorphosis of anuran amphibians, a profound reorganization occurs in the larval immune system as the tadpole changes its physiology, morphology and behavior. This reorganiza-

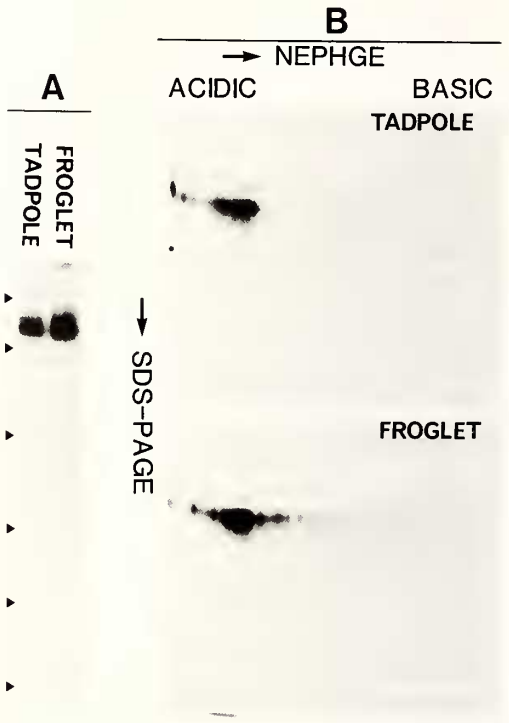

FIG. 1. Analysis of tadpole and froglet XTLA–1 molecules by SDS–PAGE (A) and O'Farrell's two-dimensional gel electrophoresis (B). Thymocytes from J strain tadpoles and froglets were radioiodinated, solubilized with lysis buffer containing 1% Nonidet P–40, and immunoprecipitated with XT–1 monoclonal antibody followed by protein A-Sepharose CL–4B beads. For SDS–PAGE, immunoprecipitates were boiled in SDS sample buffer containing 5% 2-mercaptoethanol and electrophoresed on 10% polyacrylamide gels. For two-dimensional gel electrophoresis, immunoprecipitates were deglycosilated with endo F glycosidase, and separated by nonequilibrium pH gel electrophoresis (NEPHGE) on first dimension. Electrophoresed tube gels were immersed in SDS sample buffer and then subjected to second dimension SDS–PAGE. Electrophoresed gels were visualized by autoradiography on X-ray films. Molecular size standards (150 KD, 94 KD, 67 KD, 43 KD, 30 KD and 20.1 KD) were run on the same gels and indicated with arrowheads in (A).

tion is suggested to involve a nearly complete replacement of lymphopoietic cells and a reorganization of the microenvironment where lymphocytes differentiate [2]. Thus, lymphopoietic cells in the early-larval thymus are replaced by precursor cells derived from a distinct compartment of the embryonic dorsal lateral plate mesoderm [9], so that the reconstituted lymphopoietic system may supply the froglet with adult type lymphocytes having "mature" immunological functions. Recently, it was demonstrated that the MHC class I molecules are not expressed on the lymphocyte surface until the metamorphic climax [10]. Although their function is remained to be clarified, the difference in two-dimensional gel patterns of the XTLA-1 molecules found in the present study might provide, as the MHC class I antigens, a marker to distinguish adult type T cells from larval type ones in *Xenopus*. Such a marker should prove useful in studying the cellular basis of the development of immune reactivity and tolerance during amphibian metamorphosis.

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